WS21.1 Cystic fibrosis incidence in Portugal

P. Pacheco[1], C. Silva[1], P. Loureiro[1]. *[1]Instituto Nacional de Saúde Dr. Ricardo Jorge, Genética Humana, Lisboa, Portugal*

Objectives: The incidence of Cystic Fibrosis (CF) in the European Caucasian population is about 1 in 2500 live births and about 1 in 25 people are carriers of a CF genetic mutation. These numbers were generally adopted, but there may be marked regional variations. The p.Phe508del mutation is the most common mutation causing CF. In central and northern Europe, this mutation has a frequency of about 70%. But previously published data indicated that in Spain and Portugal the frequency of this mutation was much lower, around 50%. To estimate the incidence of CF in Portugal we have determined with high accuracy the frequency of the p.Phe508del in our CF patients and calculate the incidence of the disease by determining the incidence of CF carriers analysing consecutive new-borns.
Methods: A group of 509 CF patients, with extensive or complete CFTR gene analysis (by DGGE and/or sequencing), was clinically revaluated and part of the diagnostics were excluded. The frequency of the p.Phe508del mutation was determined in the confirmed CF cases as 66.4%±3.2% (95% CI). Then, we have analysed 1603 Guthrie-cards and detected 26 p.Phe508del carriers, and a CF carrier incidence of 2.44%±0.75% (95% CI) (sample size calculator). About 1 in 37 people are CF carriers and the CF incidence is 1 in 5500.
Conclusion: The frequency of the p.Phe508del in Portuguese CF patients 66.4% is similar to the French 67.2% and other European countries, but different from the reported data of Spain 50.6%. The CF incidence in our population of 1 in 5500 is much lower than expected, probably due to our population admixture.

WS21.2 Non-invasive prenatal diagnosis (NIPD) of cystic fibrosis by quantitative real time mutant enrichment with 3′-modified oligonucleotides (MEMO) PCR

C. Guissart[1], V. Debant[1], C. Bareil[1], V. Viart[1], M. des Georges[1,2], M. Claustres[1,2], M.C. Vincent[1,2]. *[1]CHRU Montpellier, Laboratoire de Génétique Moléculaire – IURC, Montpellier, France; [2]U827_INSERM, Laboratoire des Maladies Rares, Montpellier, France*

In the recent years, NIPD has found new applications in monogenic diseases diagnostic for paternally inherited mutations. Such an approach remains very challenging because of the inherent limitations of molecular testing methods, the low concentration of circulating free fetal DNA (cff-DNA) in maternal plasma and the "maternal contamination".
We developed and evaluated an NIPD test for Cystic Fibrosis for the most common French mutation. It consists in searching the paternal mutation in compound heterozygous families using MEMO-PCR method (Lee et *al* 2011) associated with real-time PCR. The analytical validation step was made on chimeric DNA control samples that contain 0%, 2%, 5%, 10%, 50% and 100% of mutant DNA at a final concentration of 100 pg/μL. In addition, we have assessed a mini STR kit as quality control to confirm the presence of cff DNA in the maternal plasma. We obtained the first proofs of concept of our approach by the accuracy for the detection of the p.Gly542* mutation. Real-time MEMO PCR demonstrated the blocking efficiencies of normal allele with an enrichment of mutant allele and reveals differences in melting curve shape that correlate with the nature of each chimeric sample with a detection threshold of 2%. A tri-allelic profile was found for all tested maternal plasma.
Our new approach offers numerous advantages: it is simple, cost and time efficient, and applicable to different type of variants: point mutations or small insertion/deletions. Therefore, this method does not require complex equipment or bioinformatics setting and can be easily applicable in routine. Nevertheless validation studies are necessary to be manageable in clinical practice.

WS21.3 Clinical variability in patients with cystic fibrosis and D1152H mutation

V. Terlizzi[1], R. Ingino[2,3], A. Elce[3,4], A. Tosco[1], F. Improta[1], N. Cirilli[5], R. Gagliardini[5], D. Salvatore[6], V. Carnovale[7], M.A. D'Agostino[7], A. Sepe[1], N. Amato[1], F. De Gregorio[1], A. Casale[1], V. Raia[1], G. Castaldo[3,4]. *[1]Department of Pediatrics, University of Naples Federico II, Cystic Fibrosis Center, Naples, Italy; [2]CEINGE Advanced Biotechnology, University of Naples Federico II, Naples, Italy; [3]Department of Medical Biochemistry and Biotechnology, University of Naples, Federico II, Naples, Italy; [4]CEINGE Advanced Biotecnology, University of Naples Federico II, Naples, Italy; [5]CF Centre, United Hospitals, Ancona, Italy; [6]Cystic Fibrosis Centre, AOR Ospedale San Carlo, Potenza, Italy; [7]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Adult Unit Cystic Fibrosis Center, Naples, Italy*

Objectives: D1152H is a IV class mutation causative of milder clinical manifestations in Cystic Fibrosis (CF). In order to assess the prevalence and clinical features of patients (pts) with CF and D1152H mutation, we retrospectively evaluated a cohort of patients in follow-up at 3 CF Centers.
Methods: We analyzed age and symptoms at diagnosis, sweat test chloride (ST), sputum, radiological exams, pancreatic status, FEV1% predicted and complications in 630 CF pts (mean age: 20.6 yrs, range 4 months-49 yrs). D1152H mutation was detected in 20/630 pts (3.1%; mean age: 19.8 yrs, range 3–49 yrs); 14/20 were compound heterozygous for other CFTR causing mutations. Mean age at diagnosis of this subset of pts was 12.6 yrs (range: 6 months-48 yrs). 3/20 were diagnosed by neonatal screening, 6/20 by respiratory symptoms, 4/20 by infertility, 4/20 by familiarity, 2/20 by recurrent pancreatitis and 1/20 by intestinal symptoms. 3/20 pts had pancreatic insufficiency. At diagnosis the ST was negative/borderline in 18/20 cases. 4/20 suffered from recurrent pancreatitis. 9/20 showed bronchiectasis, only 5/20 have occasional *Pseudomonas aeruginosa* (PA) infections during the follow up while 2/20 had chronic infection by PA. 6/20 suffered from complications as chronic pancreatitis (1/20), hemoptysis (2/20), liver disease (2/20) and allergic bronchopulmonary aspergillosis (1/20).
Conclusions: Although D1152H mutation is related to milder CF forms our results show a variability of clinical symptoms, including early onset of lung disease. The inclusion of D1152H mutation in screening panels could be recommended in order to early perform therapeutic interventions.

WS21.4 Clinical and morphological characteristics of sporadic genetically determined pancreatitis compared with idiopathic pancreatitis: Higher risk of pancreatic cancer in CFTR variants

X. Pepermans[1], C. Hamoir[2], H. Piessevaux[2], A. Jouret-Mourin[3], B. Weynand[3], J.-B. Habyalimana[2], A. Geubel[2], J.-F. Gigot[4], P.H. Deprez[2], T. Leal[5]. *[1]Center for Human Genetics, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium; [2]Hepato-Gastroenterology Department. Cliniques Universitaires Saint-Luc. Université Catholique de Louvain, Brussels, Belgium; [3]Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Pathology Department, Brussels, Belgium; [4]Department of Abdominal Surgery and Transplantation. Cliniques Universitaires Saint-Luc. Université Catholique de Louvain, Brussels, Belgium; [5]Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Université Catholique de Louvain, Brussels, Belgium*

Idiopathic pancreatitis is a multigenic and multifactorial disease. Genetically determined pancreatitis is associated with *PRSS1*, *SPINK1* and *CFTR* mutations.
This study aims at investigating clinical and morphological characteristics of patients diagnosed with genetically determined sporadic pancreatitis. Inclusion criteria were the presence of *PRSS1*, *CFTR* or *SPINK1* mutations in patients with idiopathic or chronic pancreatitis. Patients with hereditary pancreatitis were excluded. Age- and gender-matched patients with idiopathic pancreatitis and negative genetic testing served as controls (n = 68).
Genetic testing was performed in 351 referrals to our centre since 1999. Of these, 61 (17.4%) carried at least one mutation (34 *CFTR*, 10 *PRSS1* and 13 *SPINK1*), while four showed a combination of mutations. Follow-up has been extended to a median of 5 (range 1–40) years. Similar clinical features were noted in cases and controls except for an earlier age of onset of pancreatic symptoms and a higher incidence of pancreatic cancer in patients with *CFTR* mutations compared with controls ($p < 0.05$). The ratio of observed to expected pancreatic cancers averaged 26.5 (95% CI: 8.6–61.9). All pancreatic cancer patients were smokers.
Except for an earlier age of onset of pancreatic disease, clinical features of patients with sporadic idiopathic pancreatitis and gene mutations were similar to those of controls. A significantly higher occurrence of pancreatic cancer was observed in patients carrying *CFTR* mutations. We therefore suggest including patients with CFTR variants and risk factors into a screening and surveillance program, while strongly advising them to stop smoking.